



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

Date: March 20, 2017

SUBJECT: New Tolerance Enforcement Methods for the Determination of Residues of Quinclorac and its Metabolite Quinclorac Methyl Ester in/on Plant Commodities.

PC Code: 128974

DP Barcode: D438546

CAS No.: 84087-01-4

Case No.: 7222

MRID No.: 49133401, 49133402

40 CFR: §180.463

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Introduction

BASF Canada Inc. submitted high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods, D9708/02 for the determination of residues of quinclorac and D9806/02 for the determination of quinclorac methyl ester in crop matrices—cereal grain (wheat) and oil seed (canola). Both methods were jointly reviewed previously by the Agency and PMRA of Canada and found to be acceptable for data collection (MRID 49133402, cited in DP#s 402192/412463, 9/30/13, S. Hummel). Both methods have an LOQ of 0.05 ppm and an LOD of 0.01 ppm. Both methods monitor two ion transitions. The methods have been validated by the independent laboratory study (JRF America). Method D9708/02 and Method D9806/02 are a great improvement over previous enforcement analytical method, the GC/ECD method that requires the use of toxic chemical diazomethane for the methylation step. The purpose of this MEMO is to concur with the company's proposal that BASF Method D9708/02 for quinclorac and BASF Method D9806/02 for quinclorac methyl ester are the appropriate tolerance enforcement methods.

Discussion

Method D9708/02 and Method D9806/02 have been used successfully for data collection in the storage stability study of quinclorac and its metabolite quinclorac methyl ester in canola (MRID # 49133402). The methods are discussed below.

Method D9708/02 for Quinclorac

Briefly, crop samples (e.g., canola seed and meal samples) were extracted with NaOH and acetone. Residues were cleaned up by liquid-liquid partitioning with saturated sodium chloride (NaCl) solution and dichloromethane (DCM). The DCM layer was discarded. The residues in the aqueous phase were then acidified (pH ~2-3) with formic acid, partitioned into DCM and evaporated to dryness. The residues were re-dissolved in acetonitrile:water (10:90, v/v), filtered and analyzed by LC/MS/MS. For **canola oil**, the sample was dissolved in hexane. Residues of **quinclorac** in the organic (hexane) layer were diluted with acetonitrile:0.1 N NaOH (1:1, v/v), followed by methanol, shaken and centrifuged, and the residues in the aqueous acetonitrile layer were, after adjusting with acetonitrile:0.1 N NaOH (1:1, v/v) solution, combined with an aliquot of the original aqueous extracts, as described above. The residues were subjected to liquid-liquid partitioning with DCM and evaporated to dryness. The residues were re-dissolved in acetonitrile:water (10:90, v/v), filtered, and then analyzed by LC/MS/MS.

Quinclorac residues in canola seed and oil were quantitated by liquid chromatography (LC) method with tandem mass spectrometry (MS/MS) detection, monitoring ion transitions from m/z 242 to 160.8. The limit of quantitation of the method was reported as 0.05 ppm for quinclorac in each matrix. The concurrent recoveries for quinclorac in all matrix at spiking level of 0.5 ppm were within the acceptable range of 70-120%.

Method D9806/02 for Quinclorac Methyl Ester

Briefly, residues of **quinclorac methyl ester** in/on **canola seed** and meal samples were extracted with acetone. An aliquot was taken and evaporated to dryness. The residues were cleaned up by liquid-liquid partitioning in saturated NaCl solution and DCM. The residues in the DCM layer were evaporated to dryness, re-dissolved in methanol:water solution (1:1, v/v), filtered, and then analyzed by LC/MS/MS. For **canola oil**, samples were extracted with a mixture of hexane, acetonitrile:water (2:1, v/v), and methanol. The residues of **quinclorac methyl ester** in the aqueous acetonitrile layer were diluted with acetonitrile:water (2:1, v/v), an aliquot was taken, further diluted with methanol:water (1:1, v/v), filtered, and analyzed by LC/MS/MS.

Quinclorac methyl ester residues in canola seed and oil were quantitated by liquid chromatography (LC) with tandem mass spectrometry (MS/MS) detection, monitoring ion transitions from m/z 256 to 224. The limit of quantitation of the method was reported as 0.05 ppm for the metabolite quinclorac methyl ester in each matrix. The concurrent recoveries for quinclorac methyl ester in all matrix at spiking level of 0.5 ppm were within the acceptable range of 70-120%.

Independent Laboratory Validation

An independent laboratory method validation (ILV) was conducted by JRF America to verify the reliability and reproducibility of the LC/MS/MS BASF Method D9708/02 for the determination of residues of quinclorac in wheat grain and canola seeds and to verify the reliability and reproducibility of the LC/MS/MS BASF Method D9806/02 for the determination of residues of the metabolite quinclorac methyl ester in canola seeds (MRID # 49133401).

BASF Method D9708/02 as described in the ILV report:

Residues of quinclorac are extracted from plant sample with a mixture of acetone and 0.1 N NaOH (3:1, v/v) using a polytron. An aliquot of the extract is concentrated to an aqueous phase by removing acetone under nitrogen evaporation. The aqueous phase is subjected to liquid-liquid partition using dichloromethane. The resulting aqueous phase is then acidified to pH 2-3 and re-partitioned with dichloromethane. After partition, an aliquot of dichloromethane is evaporated to dryness and then re-dissolved in acetonitrile:water (10:90, v/v). Quinclorac residues are determined using LC/MS/MS. The ion transitions monitored were m/z 242 \rightarrow 161 and m/z 242 \rightarrow 224; m/z 242 \rightarrow 161 was proposed as transition quantification. The method limit of detection (LOD) was reported as 0.01 ppm and the method LOQ was reported as 0.05 ppm for quinclorac residues in plant matrices.

BASF Method D9806/02 as described in ILV report:

Residues of quinclorac methyl ester are extracted from plant sample with acetone using a polytron. An aliquot of the extract is evaporated to dryness under nitrogen evaporation. The dry residue is re-dissolved in saturated sodium chloride and the resulting solution is subjected to liquid-liquid partition using dichloromethane. After partition, an aliquot of dichloromethane is evaporated to dryness and then re-dissolved in methanol:water (1:1, v/v). Quinclorac methyl ester residues are determined using LC/MS/MS. The ion transitions monitored were m/z 256 \rightarrow 224 and m/z 256 \rightarrow 161; m/z 256 \rightarrow 224 was proposed as transition quantification. The method LOD was reported as 0.01 ppm and the method LOQ was reported as 0.05 ppm for quinclorac methyl ester in plant matrices.

The methods were validated on control wheat grain and canola seed samples for method D9708/02 (quinclorac) and on control canola seed samples for method D9806/02 (quinclorac methyl ester). Samples were spiked with either quinclorac or quinclorac methyl ester at the LOQ (0.05 ppm) and at the 100 x LOQ (5.0 ppm) following the method protocols. Residues of quinclorac and quinclorac methyl ester were quantitated by external standards. Calibration curve was linear over the range of 0.1 - 10.0 ng/mL ($r > 0.996$).

The validations of method D9708/02 in wheat grain and canola seeds and method D9806/02 in canola seeds were successful. The average recoveries ranged from 84% to 91% for both primary and confirmatory ions. There was only one recovery obtained outside the acceptable range of 70-120% (67% in confirmatory transition; canola seed spiked at 0.05 ppm with quinclorac methyl ester).

The independent laboratory study confirmed the validity of BASF analytical method D9708/02 for the determination of quinclorac and BASF analytical method D9806/02 for the determination of quinclorac methyl ester in plant matrices. These LC/MS/MS methods are suitable as enforcement methods in plant matrices.

Conclusion

BASF Method D9708/02 (for quinclorac) and BASF Method D9806/02 (for quinclorac methyl ester) were previously reviewed jointly by the Agency and PMRA. They were found to be acceptable for data collection and tolerance enforcement of residues of quinclorac and its methyl ester metabolite in/on plant commodities. D9708/02 and D9806/02 employ LC/MS/MS method without the use of toxic chemical diazomethane for the methylation step. The methods are further revised to eliminate a clean-up step by a C₁₈ SPE column. Method D9708/02 and Method D9806/02 are a great improvement over previous enforcement analytical method (GC/ECD method) that requires a methylation step. The validated LOQ for both methods is 0.05 ppm, and LOD 0.01ppm. Both methods monitor two ion transitions.

Based on the submitted independent laboratory validation results, HED is convinced that BASF analytical method D9708/02 for the determination of quinclorac and BASF analytical method D9806/02 for the determination of quinclorac methyl ester are adequate for tolerance enforcement. HED will send to the Analytical Chemistry Branch Laboratory of the Biological and Economic Analysis Division an electronic copy of MRID # 49133401 that describes BASF Method D9708/02 for quinclorac and BASF Method D9806/02 for quinclorac methyl ester.